Author's response to reviews

**Title:** Effective Dosing of L-Carnitine in the Secondary Prevention of Cardiovascular Disease: a Systematic Review and Meta-Analysis

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**Author's response to reviews:** see over
Dear Editor(s):

After feedback from your reviewers, I wish to re-submit the following manuscript entitled “Effective Dosing of L-Carnitine in the Secondary Prevention of Cardiovascular Disease: a Systematic Review and Meta-Analysis” pursuant to publication as an Article in *BMC Cardiovascular Disorders*. Each of the three reviewers’ comments and responses thereto are detailed below and have been addressed in the revised manuscript. We have also provided a track-changed version of the revised manuscript for the editors' and reviewer's convenience.

Thank you for your time and consideration. I look forward to hearing from you.

Kind regards,

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Reviewer: Edgars Liepins

Authors state: "Cardiac muscle cannot synthesize carnitine de novo and must acquire carnitine exogenously via carnitine-acylcarnitine carrier (CAC), a carrier protein that imports fatty acyl moieties into cardiac mitochondria for #-oxidation, the primary energy source in heart muscle." However, the first part of following sentence is about exogenous carnitine, which is transported in cell by OCTN2 and a second part is related to mitochondria and there indeed is the CAC transport system. The sentence must be divided or modified accordingly.

Response: We have made the recommended change to the revised Introduction.

Add citations or change the sentence if no appropriate studies: "Thus, deficiencies in carnitine or its transporter CAC have particularly adverse effects on cardiomyocytes, resulting in cardiomyopathy, cardiac arrhythmia, cardiac insufficiency, and heart failure."

Response: We have added the relevant citation to the revised Introduction.

Authors write: "Following ingestion of a dietary L-carnitine supplement, the rate of L-carnitine excretion increases rapidly, and the efficiency of L-carnitine reabsorption decreases as the filtered load of L-carnitine increases above normal (8)." However, it's not fully correct. Excretion increases not because reabsorption decreases, but because absolute reabsorption does not changes. As it correctly stated in the discussion (actually repetition of introduction), in general reabsorption depends on the number of the OCTN2 in the distal tubule. This amount of transporters ensures about the 20-60uM in plasma concentration of carnitine in the blood. As carnitine does not significantly influence expression of OCTN2, there are no changes in the level of absolute carnitine reabsorption. Nevertheless, same amount of OCTN2 could not transport more carnitine in case of increased amount of content in glomerular filtrate and therefore most of the carnitine above optimal concentration (40-60 uM in the plasma) is eliminated via urine. Overall mentioned sentence should be changed. Please consider keep all statements about carnitine transport in introduction.

Response: We have made the recommended change to the Introduction and redacted this issue from the revised Discussion.

As the authors mention at the beginning of the introduction, carnitine action is related to intracellular (mitochondrial) processes of fatty acid transport. However, in the manuscript (either introduction or discussion) is not mentioned that none of intervention clinical studies have not measured carnitine in muscles. Also is known that after carnitine treatment, compared to plasma concentrations, in the muscles even less changes in carnitine content were observed. In addition, carnitine content in muscles about 2 times exceed content necessary for mitochondrial transport of fatty acids.

Response: We have extensively addressed the issue of skeletal muscle carnitine in the revised Discussion.

Authors mention that there the bioavailability of supplemented L-carnitine ranges only from a mere 5-18%. It is known that large doses of carnitine are converted to TMA by intestinal microbiota. Recently has been published studies describing adverse effects related to carnitine conversion by microbiota to TMA and subsequently to TMANO which has pro-atherogenic action. Authors have to discuss these effects, which could mask potential benefit of oral carnitine supplementation.
Response: We have extensively addressed the issue of L-carnitine's conversion to TMA by intestinal microbiota in the revised Discussion.

As I mentioned before, authors write in the introduction: "Thus, deficiencies in carnitine or its transporter CAC have particularly adverse effects on cardiomyocytes, resulting in cardiomyopathy, cardiac arrhythmia, cardiac insufficiency, and heart failure." The discussion could be also greatly improved if at least several non-interventional studies will be cited (Liepinsh E et al. Nutr Res. 2012; Ueland T et al 2013, Int J Cardiol.; Koeth RA, 2013, Nat.Med.; etc.) and contradictory effects will be discussed.

Response: We have discussed these recent studies concerning the adverse CVD effects of L-carnitine supplementation in the revised Discussion.
Reviewer: Nikolaos Papageorgiou

-The number of studies included in the present analysis is small as well as the number of the patients enrolled in the majority of these studies.
-There are several studies/trials after 2012 which have not been reported.
-The authors should have reported also other studies with longer follow up period as those after 2012.

Response: After an exhaustive search of the literature according to the inclusion and exclusion criteria, these five studies were the only trials remaining for inclusion in this meta-analysis. That being said, we found low heterogeneity between the five trials in all-cause mortality ($I^2=22\%$) (Fig. 2) and detected no heterogeneity between the two trials reporting heart failure ($I^2=0\%$), unstable angina ($I^2=0\%$), and myocardial reinfarction ($I^2=0\%$). This limitation is stated in the Discussion.

-It would be really interesting analyzing and reporting the different types of arrhythmias post MI.

Response: Due to lack of reported data, we could not analyze L-carnitine dosing in the secondary prevention of ventricular arrhythmia in the setting of acute myocardial infarction. This limitation is stated in the Discussion.

-I would suggest further dose-based analysis (wide range).

Response: Due to the limited dosing range in the included studies, only the effects of the four daily oral maintenance dosages (2 g, 3 g, 4 g, and 6 g) were analyzed here. Therefore, we could not ascertain whether daily oral maintenance doses under 2 g or above 6 g are equally effective, nor did we examine the effects of different initial loading administrations. This limitation is stated in the Discussion.

-It is really important to analyze the dietary patterns and mention if there is repletion to L-carnitine/or to anyone of its metabolites. Could the effects of L-carnitine be suppressed by the different dietary patterns or medication?

Response: Due to lack of reported data, we could not analyze the dietary patterns or medication usage in this meta-analysis, which may have had differential effects on the metabolism of L-carnitine. This limitation is stated in the Discussion.
"The interaction test yielded no significant differences between the effects of the four daily maintenance dosages of L-carnitine (i.e., 2 g, 3 g, 4 g, and 6 g) on all-cause mortality (RR=0.77, 95% CI [0.57-1.03], P=0.08) (Fig. 2)." This would be considered a trend for benefit (i.e. 0.05-0.09). This should be explained as a trend. Also, what does this trend indicate? Is it that 6 grams has a trend for reducing mortality vs. 2?? This needs to be further explained – rather than simply stating "statistical insignificance."

Response: Thank you for the insightful comment Dr. DiNicolantonio – I completely overlooked this important finding. Analysis of the all-cause mortality risk ratios for each dosage yielded a statistically insignificant trend favoring the 3 g dose (RR=0.48) over the lower 2 g dose (RR=0.62), which was favored over the higher 4 g and 6 g doses (RR=0.78, 0.78). Although a statistically insignificant trend, this profile creates a bell-shaped curve with the 3 g dose as the optimal dosage in terms of all-cause mortality.

Therefore, the conclusion of the study now states: “there appears to be no significant marginal benefit in terms of all-cause mortality, heart failure, unstable angina, or myocardial reinfarction in the setting of acute MI for oral L-carnitine maintenance doses of greater or less than 3 g per day.”